FEEDING HABITS OF CRANGONID SHRIMPS AND SOME ASPECTS OF SEDIMENT-DETRITAL FOOD SYSTEMS IN LOWER COOK INLET, ALASKA

A

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FEEDING HABITS OF CRANGONID SHRIMPS AND SOME ASPECTS OF SEDIMENT-DETRITAL FOOD SYSTEMS IN LOWER COOK INLET, ALASKA

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ABSTRACT

The relationship of crangonid shrimps with the sediment-detrital food system in Cook Inlet, Alaska, was investigated. Prey items included small crustaceans (35% frequency of occurrence) and polychaetes (24% frequency of occurrence). Inorganic sediment averaged 55% of the stomach contents. Sediment contained up to 14.5 mg organic carbon g^{-1} sediment. Respiration rate of *Crangon dalli* averaged 25.7 µl O_2 g^{-1} hr^{-1}. The daily percent energy potential available to crangonids from sediment-detrital and bacterial carbon ranged from 4.3% to 18.6% for sediment total organic carbon and up to 5.3% for the bacterial carbon fraction. A maximum potential of 40% of daily energy needs from sediment total organic carbon and 17.4% from bacterial carbon was calculated. Sediment carbon may be supplemental to an opportunistic feeding style enhancing the shrimps' ability to survive in a range of habitats. Feeding habits of crangonid shrimp and sediment-detrital quality was related to oceanographic conditions.

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INTRODUCTION

Sediment-detrital food systems are currently an area of intensive research (Baker and Bradnam, 1976; Fenchel and Jørgensen, 1977; Kofoed, 1975, Rieper, 1978; Tenore, 1975; 1977). A significant number of benthic dwelling invertebrates meet their energetic needs by ingestion of sediment enriched with bacteria and detritus to varying degrees. Of interest is the importance of bacteria in the nutrition of these detrital feeding animals. For instance, it has been demonstrated that some detrital feeding animals assimilate estuarine detritus and its associated bacteria (Adams and Angelovic, 1970) and growth efficiency is in some cases higher when bacteria enriched food sources are included in the diet (Kofoed, 1975). Further, some authors feel that bacteria may play a central role in the energetic pathways of these food systems (Fenchel and Jørgensen, 1977).

It is of interest to examine the dynamics of detrital food systems in oil and natural gas producing areas, such as Cook Inlet, Alaska, where hydrocarbons may become associated with sediments. Oil and gas exploitation and related potential disturbances in Alaska waters has led to a series of studies by the Outer Continental Shelf Environmental Assessment Program (OCSEAP). In Cook Inlet, Alaska, studies have established baseline data on distribution, abundance, and trophic relationships of nearshore benthic communities (Feder *et al.*, 1980), microbial activity (Griffiths and Morita, 1979), and sedimentation characteristics (Larrance, 1979).

The Study Area - Cook Inlet, Alaska

Cook Inlet is a positive, partially-mixed estuary located north of the Gulf of Alaska in the southcentral portion of the state in an area surrounded by mountains and glaciers (Figure 1). The inlet is some 370 km long in a northeast-southwest direction, and 139 km in width at the mouth. The average depth is approximately 60 m with depths to 200 m at the mouth. For the purposes of this report, "lower" Cook Inlet encompasses the area from Cape Douglas in the south to Chinitna Bay in the north. Other authors typically consider the forelands region as the northern limit of "lower" Cook Inlet (Burbank, 1974).

Circulation in the inlet has been previously described (Burbank, 1977). Circulation is thought to be primarily tidal, modified by the Coriolis effect and morphology of the basin. Currents to 6.5 kts may be generated by tides. Oceanic water enters the inlet from the east via the Alaska Current. Water is carried into the inlet on flood tides and flows north along the eastern half of the upper inlet. On the ebb tide relatively fresh silt-laden water from the upper inlet is carried out along the western shores. Incoming oceanic water has been characterized as saline ($32°/_{oo}$) relative to the out-flowing water ($28\text{-}29°/_{oo}$). The water column is well mixed along the eastern shore from the southern tip of the Kenai Peninsula north. Along the western shore the fresh water outflow stratifies on top of more saline water. Fresh water input to the inlet is supplied primarily by the Susitna River and Knik Arm at the head of the inlet. The influence of wind on the general circulation of the inlet is not fully understood. Burbank (1977) has

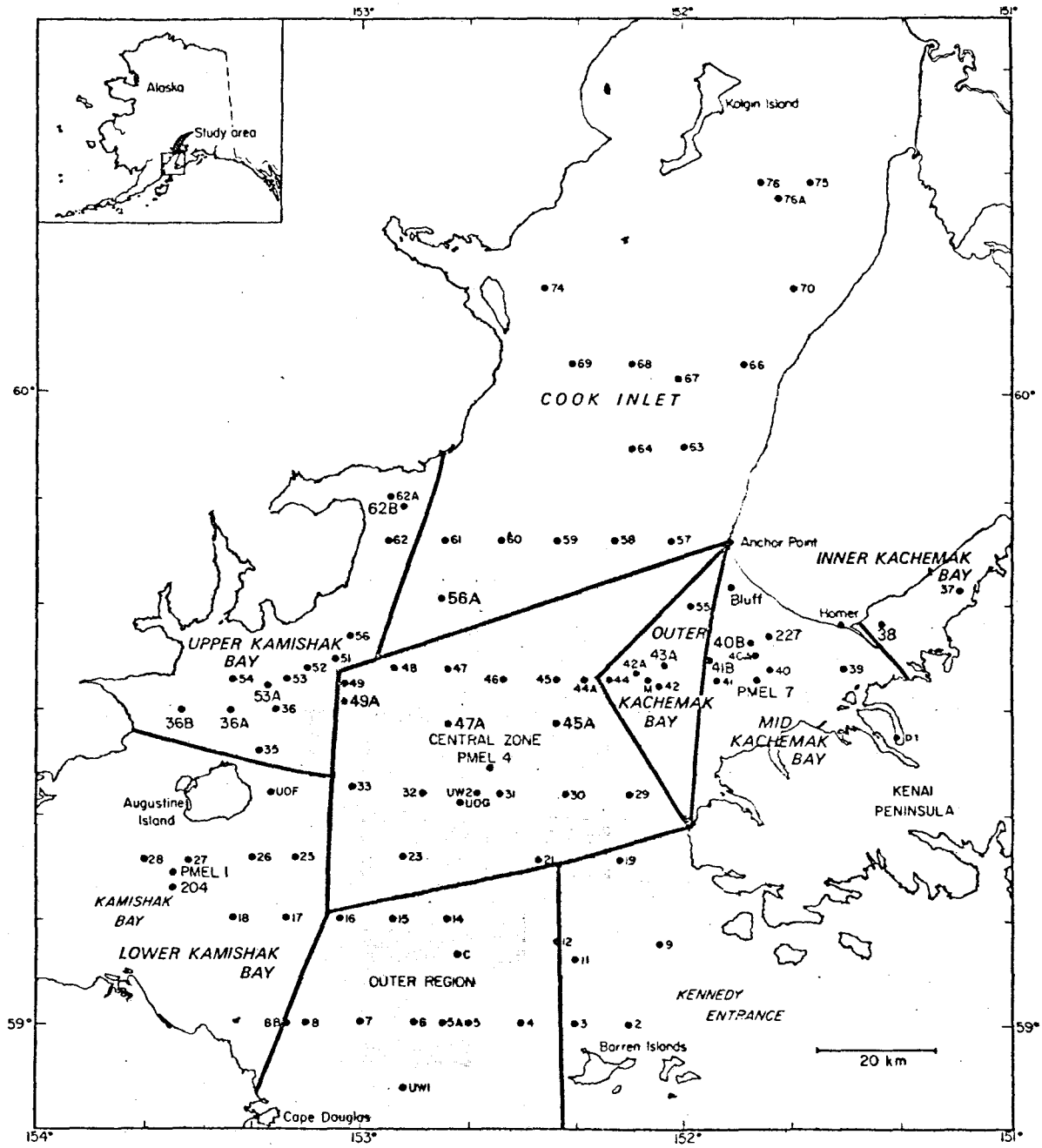

Figure 1. Lower Cook Inlet Benthic Stations. (Feder *et al.*, 1980).

prepared a generalized net surface circulation scheme in lower Cook Inlet (Figure 2).

Two important features and areas of specific interest in lower Cook Inlet are Kamishak and Kachemak Bays. These are areas of high biological activity and are important to commercial fisheries (Burbank, 1977; Crow, 1977; Feder *et al.*, 1980; Rice *et al.*, 1980). Gyre systems are present in inner and outer Kachemak Bay at least for part of the year. Eddies and gyres are also suggested in the Augustine Island, Kamishak Bay area from drift card studies, although little additional information is available for this area (Burbank, 1977). During stormy periods (September-November) the gyres may break down. Residence time of water in the outer Kachemak Bay gyre system has been estimated at 15 days, with the source of this water perhaps upwelling near Elizabeth Island. This rich water may in part account for high primary production observed in the Kachemak Bay region. Larrance (1979) reports values to 7.8 g m^{-2} day^{-1} primary production for Kachemak Bay and 6.8 g m^{-2} day^{-1} for Kamishak Bay.

Sediments in lower Cook Inlet have been characterized as facies 3, sand with variable amounts of gravel (Sharma and Burrell, 1970). It was noted that finer material is deposited in Kamishak Bay. Feder (personal communication) has noted similar patterns of patchiness of sand and clays observed in benthic grab studies. Muds and clays predominate in inner and outer Kachemak Bay, as well as in Kamishak, with coarser sands observed in middle inlet and western stations.

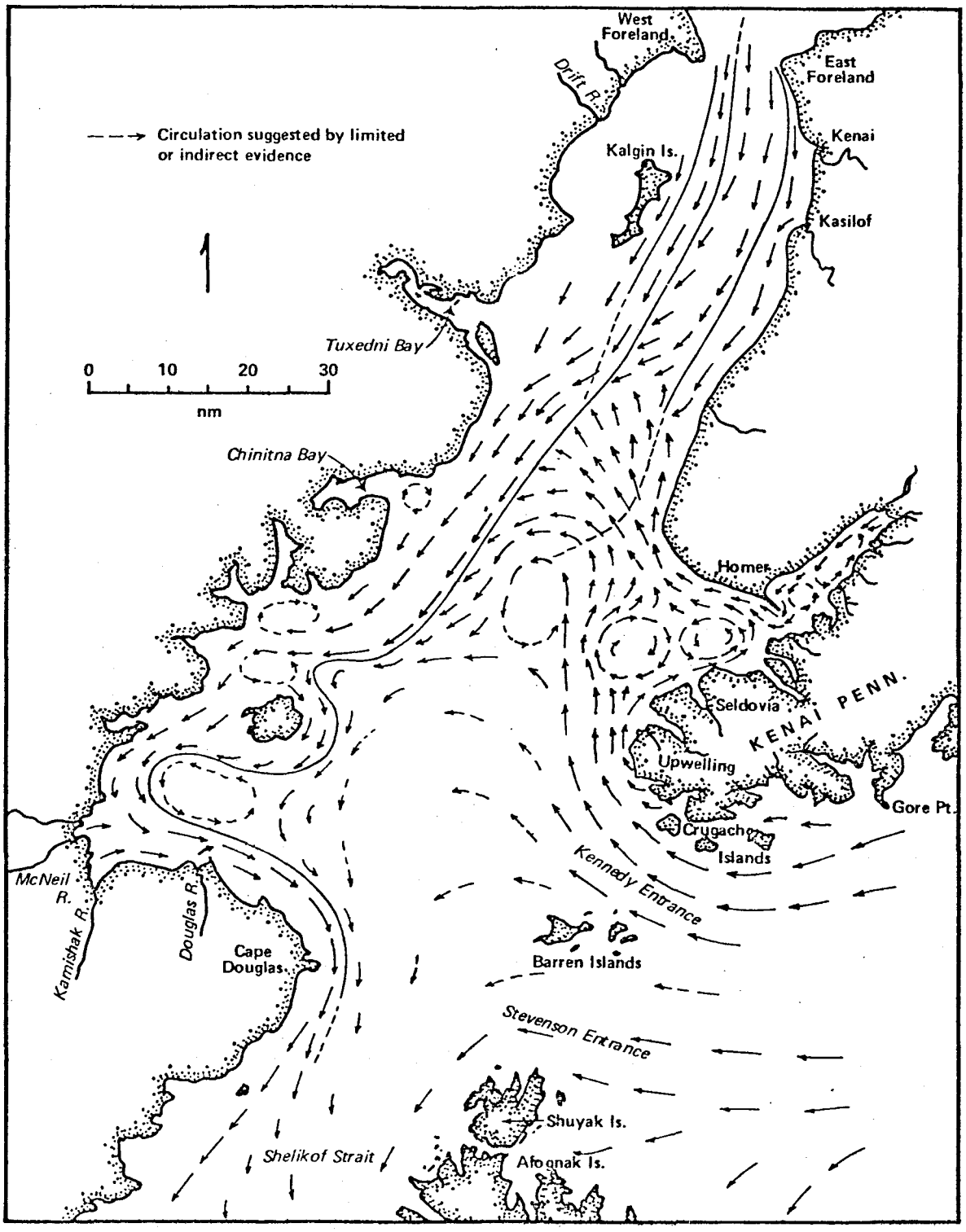

Figure 2. Net surface circulation in Lower Cook Inlet. Based primarily on data collected during the spring and summer seasons. (After Burbank, 1977.)

Potential sources of detrital inputs to the bottom include phytoplankton primary production, macroalgae, and terrestrial runoff. From phytoplankton sources, Larrance (1979) estimates 60 g C m^{-2} (Kachemak Bay), 40 g C m^{-2} (Kamishak Bay) and 17 g C m^{-2} (central inlet) were delivered to the bottom over a 4 month period. Data are not available for macrophyte inputs in lower Cook Inlet but are potentially significant in places. Mann (1972) noted the importance of macrophyte production in detritus food chains. The term detritus used in this report includes dissolved sources, egestion, secretion, etc., from within and without of the system (see Fenchel and Jørgensen, 1977; Wetzel *et al.*, 1972). The detrital food chain is then any pathway by which energy derived from detrital organic carbon becomes available to the biota.

Crangonid Shrimp

The crangonid shrimps are one of the dominant benthic invertebrates encountered in lower Cook Inlet (Feder *et al.*, 1980). In these waters there are three genera representing the family Crangonidae: *Crangon*, *Sclerocrangon*, and *Nectocrangon (Argis)*. Rathburn *et al.* (1910) give taxonomic and occurrence information for the various species in northern waters. From trawling operations in lower Cook Inlet it appears *Crangon dalli*, *Crangon franciscorum*, and *Crangon communis* are by far the most common crangonids present (Feder *et al.*, 1980). Crangonids frequently represent 20% of the animals counted in trawl catches.

Observations on trophic relationships showed crangonids to be a major food resource for many predators. Crangonids are frequently

observed in the stomachs of demersal fishes such as flathead sole, pollock, and Pacific cod (Feder *et al.*, 1980), and in snow crab (Paul *et al.*, 1979). Crangonid shrimps are considered to be important in the benthic ecosystem because of their widespread distribution and abundance and their importance as a food resource by members of the Cook Inlet food chain.

Little was known of crangonid feeding habits at the outset of this study. Initial microscopic observations of crangonid stomachs showed large amounts of sediment and detritus. Additionally, these early observations revealed that prey taken by crangonids, such as the clam *Macoma*, were often themselves sediment-detrital feeders. Crangonids may occupy a unique trophic position, perhaps serving as mediators between the sediment-detrital system and epibenthic and free-swimming predators.

To clarify the position of crangonids an investigation of the feeding ecology of these shrimp was undertaken. Specifically, the following questions were addressed.

1. What prey organisms are utilized?
2. Is sediment frequently observed in shrimp gut contents? If so, how much?
3. Is detritus a frequently observed food component?
4. What magnitude energy source does sediment-detrital and bacterial carbon represent relative to the needs of these shrimps?
5. What relationships exist between crangonid shrimp feeding habits and oceanographic characteristics of the system?

In an attempt to answer these questions, the following investigations were initiated:

1. Detailed and extensive gut sample analysis of lower Cook Inlet crangonid shrimp for frequency of prey data;
2. quantitative investigation of gut sediment content;
3. potential nutritive value of sediments, including organic carbon, and microbial biomass; and
4. metabolic rate evaluation of crangonid shrimp by measurement of respiration.

METHODS

Specimens of *Crangon dalli*, *Crangon franciscorum*, and *Crangon communis* were collected in lower Cook Inlet on six cruises from November 1977 to August 1978. Shrimp were obtained with small otter and Agassiz trawls. Depth ranged from 22 to 150 m on the stations where shrimp were taken.

For detailed gut analysis, shrimp were preserved in 10% buffered formalin. Specimens were first examined under a dissection microscope (60X) and large fragments and whole organisms identified. A subsample of the material was then placed on a slide and examined with a compound microscope (100X) and small fragments, polychaete setae, and diatoms were identified in this manner. Additional sampling with various dredges, grabs, and trawls captured potential prey organisms and aided in the identification of fragments in the shrimp stomachs.

Quantitative determination of gut sediment content was done using the IBP methodology (Holme and McIntyre, 1971). Stomach contents were dissected from preserved specimens, dried at 60°C and weighed. The sample was then treated with 10% KOH at 100°C to remove organic matter. Subsequent treatments of the sample with concentrated HCl removed chitin and shell ($CaCO_3$) fragments. The sample was again dried and weighed. The weight of the remaining inorganic material was determined by difference. Microscopic examination of the residual material after this treatment showed that the remaining material was devoid of any tissue, organic matter, or chitin fragments. The inorganic fraction is indicative of the amount of sediment ingestion. Natural carbonates associated with the sediments are destroyed by this procedure hence the actual ingestion of Cook Inlet bottom material is underestimated. A control with known amounts of sand and tissue was evaluated using the above method.

Sediment samples taken by van Veen grab were frozen and later analyzed for carbon and nitrogen content on a Carlos Erba Elemental Analyzer (Model 1104). Sediment samples were weighed on a Mettler top loading balance E200, pulverized and homogenized, sieved through a 2 mm mesh screen, and rocks removed and weighed. The sample was dried overnight in a Thelco Model 28 forced air oven at 50°C. Samples were then weighed in triplicate into tin cups on a Cahn Rg electrobalance. The sample was then combusted at 1050°C using cyclohexanone as a standard. Larrance (1979) reports that on the average, 13% of the total carbon in sediment trap samples is from inorganic sources. Using this figure,

organic carbon was computed for lower Cook Inlet stations sampled in this study.

Bacterial biomass of sediments was obtained from direct counts obtained by epifluorescence microscopy. Sediment samples (10 ml) were fixed in 1 ml of membrane-filtered (.45 µm) formaldehyde (37%). When a relatively high number of organisms was present, the samples were diluted with membrane-filtered seawater. Samples were filtered onto nucleopore filters with .2 µm pore size. The staining procedure used was that of Zimmerman and Meyer-Riel (1974). Bacterial cells were counted using a Zeiss IV Fl epifluorescence condenser microscope fitted with filters KP 500, KP 490, FT 510, and LP 520. The eyepiece used was KPT W 12.5X. Approximately 50 restriction fields were counted per sample. Only bodies with distinct fluorescence (either orange or green), clear outline and recognizable bacterial shape were counted as being bacterial cells. Using a value of 1×10^{-13} g as the amount of carbon per bacterial cell, the amount of bacterial carbon present in these sediments was calculated from the number of cells present as determined by the direct count method. Although a variety of values are available from the literature, this figure was recommended as appropriate for marine sediment mixed bacterial populations (M. J. Klug, personal communication). Bacterial carbon in 1 ml sample was converted to a dry weight basis using a factor determined in the laboratory by drying known volumes of sediments from the various stations.

Heterotrophic colony forming units (CFU's) were counted on Zobell's 2216E media containing peptone, 5.0 g; $FePO_4$, 0.1 g; yeast extract, 1.0 g;

Bacto-agar, 15.0 g; "aged" seawater, 1000 ml. Sediment was collected with a van Veen grab. In several cases plates were done from successive grabs at one station. The top 1-2 cm of sediment in a grab were removed and placed in sterile plastic bags (Nasco Whirl-Pak) until they could be processed. Plating was done at room temperature. Each dilution tube was mixed using a rotary mixer to facilitate removal of the bacteria from the sediment particles. 0.1 ml of 10^{-2} through 10^{-5} dilutions were plated. Five replicates were made at each dilution. Plates were incubated for approximately ten days after which colonies were counted using a Quebec Colony Counter. Dry sediment weights were determined by rinsing the 10^{-1} dilutions into pre-weighed beakers, drying them in a drying oven (105°C) for 24-48 hours and weighing the dry sediment. These weights were then used to calculate the viable count.

In order to evaluate the potential significance of bacterial carbon utilization relative to the metabolic demands of the animal, the base metabolic rate, or carbon demand of the shrimp was determined. This was achieved by a simple respirometer experiment. Shrimp were placed in individual flasks with 75 ml of seawater and a small amount of autoclaved sediment. Carbon dioxide evolved by the animal during the course of the procedure is trapped in 30% KOH and the Gilson respirometer measures the resultant decrease in volume. Temperature was maintained at 4.5°C during the course of the analysis (see Umbreit, 1964, for more on respirometry).

RESULTS

Food, Prey, Feeding Habits

Data are tabulated in Table 1 summarizing the frequency of occurrence information gathered on *Crangon dalli* from lower Cook Inlet. Sixty categories of food were observed in 863 individuals. The most important food items, based on the frequency of occurrence of identifiable remains in feeding shrimp, were Crustacea (unknown types) 35% frequency of occurrence, Polychaeta (unidentifiable types) 24%, Maldanidae 22%, and various types of diatoms (naviculoids 23%, Coscinodisceae 18%, and *Melosira* 26%). Unidentified organic matter (including animal tissue) was common, 32%, and sediment was observed in virtually all of the stomachs with contents, 90%. Further, 14 types of polychaete worms were identified with frequency of occurrence ranging from .4% to 10%. Unidentifiable bivalves were observed in 9% of the stomachs with contents, with 6 additional categories of identified clams infrequently observed in .6 to 1% of the samples with contents. In addition to the unidentified Crustacea 7 other crustacean categories were observed in .5 to 5% of the samples. Gastropoda 3%, Echinodermata 1%, and Porifera 2% were occasionally observed. Prey items observed in the stomach samples from the various stations typically reflect the more abundant organisms observed in grab and dredge samples at those stations. The prey observed, with few exceptions such as diatoms, are bottom dwelling organisms.

Though opportunism and generalist feeding behavior are the dominant feeding modes, active predation also took place in these animals as

TABLE 1

Food of Cook Inlet *Crangon Dalli*

Prey items / Station/Date	Depth (m)	No. Stomachs examined	No. Stomachs w/ food	Plant material	*Melosira sulcata*	Coscinodiscaceae	*Biddulphia* spp.	*Ditylum brightwellii*	Pennatae	Naviculaceae	*Nitzschia* spp.	Foraminifera	Porifera	Hydrozoa	Rhynchocoela	Nematoda	Polychaeta	Sigalonidae	Polynoidae	Phyllodocidae	Syllidae	Nereidae	Nephtyidae	Glyceridae	*Lumbrineris* spp.	Spionidae	Cirratulidae	Flabelligeridae	Scalibregmidae	Capitellidae	Maldanidae	Oweniidae	*Pectinaria* sp.	Ampharetidae	Terebellidae	*Terebellides stroemi*	Sabellidae	Gastropoda	Cephalaspidea	Bivalvia	*Nucula tenuis*	*Macoma* spp.	*Yoldia* spp.	[illegible] sp.	*Serripes* sp.	[illegible] sp.	Pycnogonida	Crustacea	Ostracoda	*Balanus* spp.	Cumacea	[illegible] spp.	Amphipoda	Ampeliscidae	Decapoda	Echinodermata	Chaetognatha	Teleostei	Unidentified animal tissue	Unidentified organic material	Unidentified calcareous material	Sediment	Pollutants (nylon fibers)
PMEL 1 06/14/78	33	50	49	11	24	10	3		7	10	2	12		3	1	31	18	4	3	4		1	7		16	8			1		8	2			1		2	1	2	1		1				2		16	1		2		1		1	1			15	2		44	
PMEL 1 07/14/78	31	50	46	9	30	16	1		5	15	5	12		1		8	25	1	11	1			5		14	7			1		18						2	10		6		2	1			1		21	2						3	2		2	25	2		40	
PMEL 1 08/15/78	33	50	39	1	10	10			1	4		13				2	20		1				3		7	6					6						2	3		18		1	1		8			21	5	1	2	1							2		1	36	1
PMEL 7 06/13/78	85	25	16	5	4	4			2	6		5	2			3	6		1				1		3	2					1							1		3		1						3	1							1		2	3	5		13	
PMEL 7 07/20/78	85	50	37	4	2	11			5	16		5	7		1	6	6		1	1			6		8	3	1			3	2			1			10		1	13		1						8	2				1		1				4	9		34	2
PMEL 7 08/14/78	85	48	34			3			5	2		1	2				4								3					2										4								7		5					3	1				14		31	
St. 37 05/16/78	31	5	2																																																				2								
St. 37 08/20/78	31	7	7				1		1							1	2								1	2					2									1								2												2		6	
St. 53 11/07/77	89	46	41	4	14	20			13	11		10	2	3		3	13		1		3		1		1	2		4			21	2			9	3	6			4	4	2	2		1	2		8	1							2	1	1	9	7	4	32	1
St. 53 06/11/78	89	18	16		6	3	1			1						2	6					1			2	1																		2				7										1	6	4		16	
St. 53 07/18/78	89	80	62	6	20	13	1	7		22		3				3	14	1	5				1	2	13	14	2		3		10		1	1	1		5			1		1	2					13			1			1	3				5	8		53	1
St. 53 08/19/78	89	50	47	2	12	13	2		3	17		3		1			7		1				1	1	7	7					10									4		3	1		24	1	1	22	3	3	3		7	3	5				3	1	2	45	
St. 62 11/14/77	27	50	27	5	2	1			1	2		2	1	1			7		4	2									2		8				1		1			2						1		7	1										8		2	25	1
St. 62 07/21/78																																																															
0400	27	25	20		4	2	1			5		1					2						2		1	2			1		3				1	2	2	1	1									6		2				2	2				3	2		20	
0800	27	25	21		5	3			1	5		1					4		1				2		1	1		1			11	1	1						2	1				1				11		8	1		2		1	1				1		20	
1000	27	25	23		8	2				3		2					6		1	1		1			2	4		2		1	6			1			1		2									7	1			1	1		2			1	2			20	1
1200	27	25	21		4	2			2	5		1		1			1		1	1			1			3					6						1	2										8		1	2	1	2	1		1			2	5		20	
1530	27	25	16		4					3		1					1		2				2		1			1			5						1		2									3				1	2		1					2	3	16	1
1900	27	25	15			2	1			3		3					1						1			1					4		1				1	1	1	2								6					1	2	1				1	2		15	
2100	27	25	10		1					1							1						1								2								1					1				4											2	4		9	2
St. 62 08/19/78	85	50	42		7	3			3	9							7		1			1			2	1					12									2		1	1		7			13				4	2	5	1	1			5	7		41	2
St. 62A tr. 7 03/ /78	27	27	23		7	1			1	4		3	1				4			2			2			1					4					1	1					3						7					1	2	2				4	4		19	
St. 62A tr. 13 03/30/78	27	32	29	4	5					4		2					5		2	1			2			1					5								2			5	1			2		10			1		2	1	2			3	5	3		29	
St. 62B 06/11/78	27	50	36		4	2				6		3		1			3		3							3					2												1					30					1		2				25	2		25	
Totals		863	678	49	173	121	11	7	50	154	7	83	15	11	2	59	163	6	39	13	3	4	38	3	82	69	3	8	8	6	146	5	3	3	13	6	35	19	13	62	4	21	10	4	40	9	1	238	17	20	12	8	24	17	32	10	1	10	129	86	12	609	10
% Frequency of occurrence (feeding shrimp)				7	26	18	2	1	7	23	1	12	2	2	.3	9	24	.9	6	2	.6	.6	6	.4	12	10	.4	1	1	.9	22	.7	.4	.4	2	.9	5	3	2	9	.6	3	1	16	6	1	.2	35	3	3	2	1	.5	3	5	1	.2	1	19	13	2	90	1
% Frequency of occurrence (total examined)				6	20	14	1	1	6	18	1	10	2	1	.2	7	19	.7	4	2	.3	.5	4	.3	9.5	8	.3	.9	.9	.7	17	.6	.3	.3	2	.7	4	2	2	7	.5	2	1	.5	4.6	1	.1	28	2	2	1	19	.4	2	4	1	..1	1	15	10	1	71	1

evidenced by type and quantity of contents in certain individual stomachs. For example, 4 intact *Nuculana* spp. (4-6 mm in size) were observed in one individual *Crangon dalli* stomach. It is noteworthy that both the high frequency of sediment and detritus, and the type of prey observed in crangonid stomachs suggest these shrimp rely heavily on the sediment-detrital food system for their nutritive needs. With few exceptions (e.g. Teleostei, diatoms, Polynoidae) the organisms utilized as food are themselves deposit feeding types such as *Lumbrineris*, Capetellidae, and *Nuculana*.

The time series sampling on Station 62 suggests the possibility of diurnal periodicity in feeding behavior. Figure 3 is a diagram of the frequency of occurrence of numbers of stomachs with and without contents in the sample with respect to time. The higher occurrence of stomachs with contents during daylight hours declining towards evening suggests the animals commence feeding at night.

Gut Inorganic Sediment Content

Data are summarized in Table 2 for the determination of quantity of sediment present in crangonid gut samples after digestion of organic matter by KOH. Samples typically contained more than 50% inorganic material on a dry weight basis. This amount, based on the contents of some 487 individuals was consistent, with the exception of samples from two stations, 40A and 18. The percentage of the sediment component estimated here is conservative, as controls with known amounts of sand and tissue showed the method underestimated sediment content 2-14%.

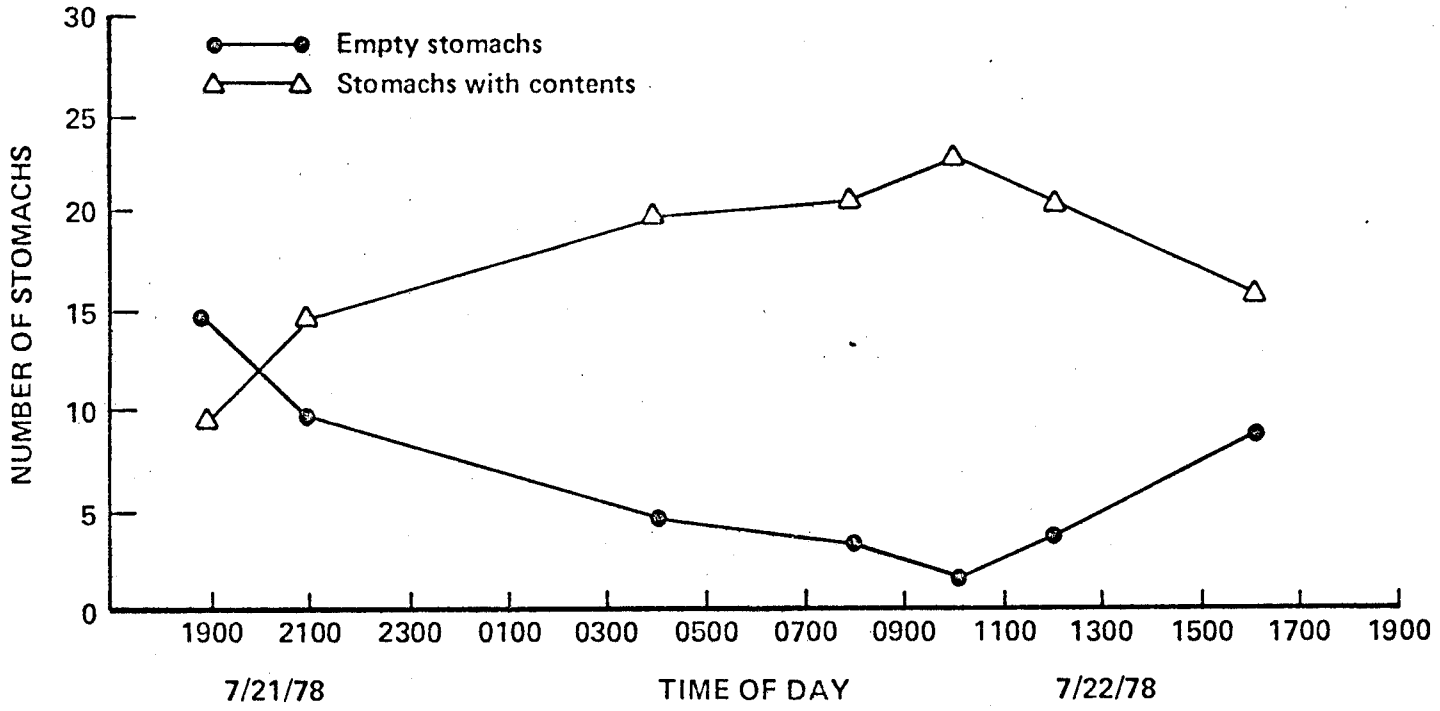

Figure 3. Feeding periodicity of *Crangon* on Station 62. Twenty-five stomachs were examined at each sampling time.

TABLE 2

Inorganic Sediment Component in Gut Contents of Lower Cook Inlet *Crangon*

Station/Animal	Date	Depth (m)	No. stomachs examined	No. stomachs with contents	dry wt. contents (g)	Dry wt. contents after KOH, HCL treatment (g)	$\bar{X}$ amt./ animal (g)[1]	% contents inorganic sediment component
40A - *Crangon dalli*	10 June 78 14 June 78	33	90	26	.699	.038	.001	5.4%
18 - *Crangon dalli*	10 June 78	53	27	15	.269	.019	.001	7.1%
35 - *Crangon dalli*	5 May 78	33	32	13	.078	.020	.002	25.6%
54 - *Crangon dalli*	14 May 78	22	91	60	.958	.907	.015	94.7%
PMEL 7 - *Crangon dalli*	20 Jul 78	85	51	35	.144	.111	.003	77.1%
PMEL 7 - *Crangon dalli*	14 Aug 78	85	178	94	.286	.179	.002	63.0%
PMEL 1 - *Crangon* spp.	20 Jul 78	33	80	51	.209	.139	.003	66.5%
62A - *Crangon dalli*	29 Mar 78	27	123	75	.651	.422	.005	64.8%
62A - *Crangon dalli*	31 Mar 78	27	72	54	.513	.238	.004	46.4%
62 - *Crangon dalli*	21 Jul 78	27	75	40	.345	.254	.006	74.0%
53 - *Crangon* spp.	11 Jun 78	89	20	13	.123	.091	.007	74.0%
27 - *Crangon dalli*	17 Jul 78	33	114	57	.423	.289	.005	70.4%

[1]The average amount of the inorganic sediment component per animal is derived by division of the dry weight of contents after KOH digestion by the number of stomachs with contents.

Microbial Biomass of Sediment

Results of the sediment microbial biomass analysis are depicted in Table 3. As indicated, there are very high numbers of microbial cells in these sediments. Sediment samples typically had 10^9-10^{10} cells ml^{-1}. In general, stations on the western side of the inlet (53, 27, 204, 62) had fewer cells than stations on the eastern side and Kachemak Bay area (PMEL 7, 37, 227).

Viable counts of some selected lower Cook Inlet stations are tabulated in Table 4. These values, ranging from 10^6-10^7 CFU g^{-1} dry weight are, as expected, lower than direct counts. On Station 37, 5.6×10^{10} cells g^{-1} were found by direct counts, compared to $\sim 1 \times 10^6$ CFU g^{-1} as determined by viable count methods. Similarly, 4.75×10^{10} cells g^{-1} (direct counts) on Station PMEL 7 is contrasted with 3.28×10^6 CFU g^{-1}.

Carbon Values of Sediments

Evaluation of total carbon content of lower Cook Inlet sediments revealed values ranging from .2 to 1.7% carbon (Table 5). On a dry weight basis, lower Cook Inlet samples contained from 1.2 (Station PMEL 4) to 16.7 (Station 40A) mg C g^{-1} sediment. Middle inlet and western stations (PMEL 4, 53, 8, PMEL 1) had less carbon than Kachemak Bay area samples (PMEL 7, 37, 40A). The computed organic carbon values range from 1.04 (Station PMEL 4) to 14.53 (Station 40A) mg g^{-1} sediment (Table 5). Kachemak Bay samples were higher in organic carbon than other regions in the inlet.

TABLE 3

Microbial Biomass of Cook Inlet Sediment Samples[1]

Station/Date	Direct Counts cells ml^{-1} sample	mg C g^{-1} sediment
53 - 18 Aug	5.0×10^9	.60
204 - Aug	1.9×10^9	.19
27 - Aug	3.9×10^9	.36
PMEL 1 - Aug	2.4×10^{10}	2.47
PMEL 1 - Aug	4.3×10^{10}	9.40
227 - Aug	3.4×10^9	.42
227 - Aug	8.0×10^9	1.00
62A - Aug	2.3×10^9	.18
62A - Aug	6.7×10^9	.53
37	4.3×10^{10}	6.20
37	3.5×10^{10}	5.10
PMEL 7 - Aug	3.8×10^{10}	4.80
PMEL 7 - Aug	4.6×10^{10}	5.80

[1]Microscopic work accomplished by research group of R. Griffiths, Oregon State Univ., Corvallis.

TABLE 4

Viable Counts of Lower Cook Inlet Sediment Samples

Station	Depth	CFU/g (x 10^6) ($\bar{X}$[1], N = 5)	S.D.[2]
8	128	3.81	.38
5	150	3.41	.77
40A-1	33	6.84	.89
40A-2	33	17.87	4.55
28-1	31	25.14	3.01
27-1	33	7.89	1.67
27-2	33	36.76	5.15
62A-1	27	3.99	.98
62A-2	27	7.07	2.31
53	89	6.02	1.01
37	31	.92	.27
PMEL 7	85	3.28	1.72
PMEL 4	65	3.87	1.67

[1]Arithmetic mean.
[2]Standard deviation.

TABLE 5

Carbon Content of Cook Inlet Sediment Samples

Sample	Wt (mg)	Total % C	Wt C (mg)	Total Wt C (mg) g^{-1} sediment	$\bar{X}^1$	Computed organic C mg g^{-1} sediment
Sta 5	4.227	.847	.0358	8.47		
	3.965	.703	.0279	7.03	8.90	7.74
	3.178	1.120	.0356	11.20		
Sta 8	2.411	.316	.0076	3.16		
	2.851	.286	.0082	2.86	2.77	2.41
	3.833	.230	.0088	2.30		
Sta 27	3.663	.884	.0324	8.84		
	2.506	.887	.0222	8.87	9.97	8.67
	3.931	1.220	.0479	12.20		
Sta 27	2.868	.426	.0122	4.26		
	3.144	.576	.0180	5.76	4.70	4.28
	3.956	.408	.0161	4.08		
Sta 28	3.933	.422	.0166	4.22		
	3.972	.471	.0187	4.71	4.44	3.86
	4.318	.440	.0190	4.40		
Sta 37	2.072	.880	.0182	8.80		
	2.685	.907	.0244	9.07	9.15	7.96
	2.476	.959	.0237	9.59		
Sta 37	2.867	.890	.0255	8.90		
	2.581	.903	.0233	9.03	8.93	7.77
	2.782	.888	.0247	8.88		
Sta 40A	4.186	1.450	.0607	14.50		
	3.315	1.580	.0524	15.80	16.70	14.53
	2.655	1.990	.0528	19.90		
Sta 40	3.916	.522	.0204	5.22		
	2.878	.457	.0132	4.57	4.89	4.25
	2.928	.489	.0143	4.89		
Sta 53	3.900	.670	.0260	6.70		
	2.824	.553	.0150	5.53	6.02	5.24
	3.047	.583	.0170	5.83		

TABLE 5

Continued

Sample	Wt (mg)	Total % C	Wt C (mg)	Total Wt C (mg) g^{-1} sediment	$\bar{X}$[1]	Computed organic C mg g^{-1} sediment
Sta 62A	3.803	.953	.936	9.53		
	2.673	1.760	.047	17.60	13.40	11.66
	3.653	1.320	.048	13.20		
Sta 62B	2.761	.549	.015	5.49		
	3.150	.403	.013	4.03	4.45	3.87
	2.722	.384	.010	3.84		
PMEL 1	3.898	.533	.021	5.33		
	3.129	.946	.030	9.46	6.83	5.94
	3.588	.571	.020	5.71		
PMEL 1	2.592	.737	.019	7.37		
	2.431	.656	.016	6.56	7.25	6.31
	2.475	.783	.019	7.83		
PMEL 4	3.315	.132	.004	1.32		
	2.940	.246	.007	2.46	1.53	1.33
	2.985	.082	.002	.82		
PMEL 4	2.128	.077	.001	.77		
	2.791	.167	.005	1.67	1.20	1.04
	2.741	.118	.003	1.18		
PMEL 7	3.464	.927	.032	9.27		
	2.655	.893	.024	8.93	9.05	7.87
	3.563	.897	.032	8.97		
PMEL 7	2.361	.377	.008	3.77		
	1.993	.432	.009	4.32	4.93	4.29
	2.547	.669	.017	6.69		
PMEL 7	2.360	1.250	.030	12.50		
	1.997	1.180	.024	11.80	11.90	10.35
	2.110	1.160	.024	11.60		
Sta 62A	2.412	1.090	.026	10.90		
	3.460	1.500	.052	15.00	11.60	10.09
	2.497	.885	.022	8.85		

[1]Arithmetic mean.

Sediment bacterial carbon values were calculated and are included in Table 3. Computed carbon from bacterial sources ranged from 0.18 (Station 62A) to 6.2 (Station 37) mg g^{-1} sediment. Kachemak Bay area samples as a result of higher direct cell counts, had more bacterial carbon.

Figure 4 depicts the relationship of organic and bacterial carbon for selected stations in lower Cook Inlet. Estimated bacterial carbon constitutes from 2% (Station 40A) to nearly 80% (PMEL 7) of the organic carbon present in the sediments. On other stations (PMEL1, PMEL 4) bacterial carbon constituted approximately 50% of the organic carbon present in the sediment samples.

Respiration, Metabolic Rate

Data from respiration rate analysis are shown in Table 6. The average respiration rate of all *Crangon dalli* measured was 25.7 µl O_2 g^{-1} hr^{-1}, with a range of 9.3 µl O_2 g^{-1} hr^{-1} to 42.7 µl O_2 g^{-1} hr^{-1}. The data displayed rather high variability, S.D. = 10.2, and respiration rate was not well correlated with shrimp size ($r = .55$, $p > .02$). By use of the ideal gas law, the average volume of O_2 uptake (25.7 µl) can be converted to moles of O_2 (1.10×10^{-6}). Multiplication by a respiratory quotient of .8 gave a mole CO_2 evolution value of 8.81×10^{-7} moles CO_2 g^{-1} hr^{-1} (Table 7). Using the molecular weight of CO_2 and the fraction represented by carbon, carbon flux due to resting metabolism in the average *Crangon dalli* was calculated:

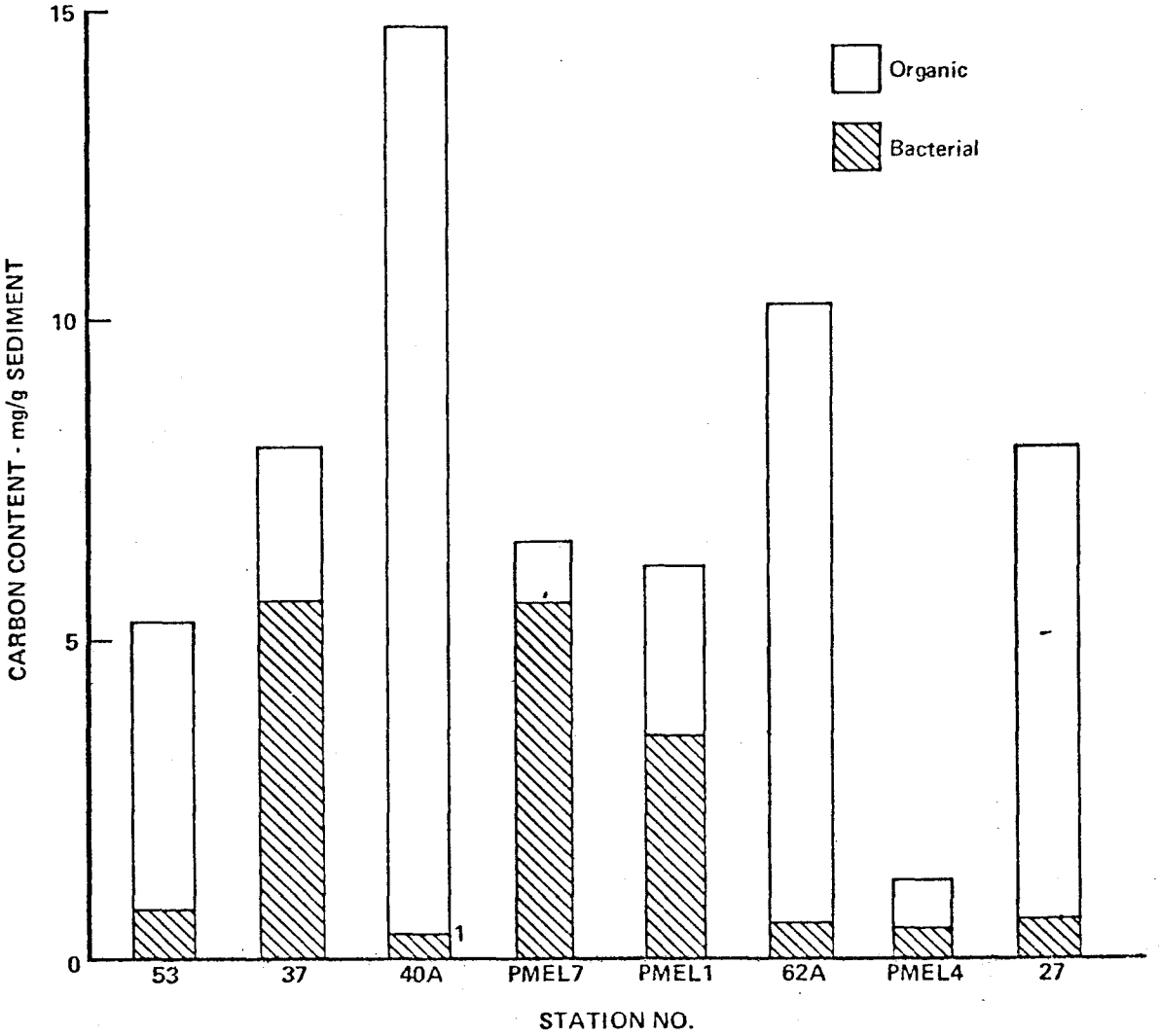

Figure 4. Relationship of bacterial and organic carbon values of sediment from selected stations in lower Cook Inlet. 1 denotes estimated from direct counts derived from viable counts.

TABLE 6

Respiration Rate of *Crangon dalli* at 4.5°C

Date	Individual shrimp wet Wt. (g)	No. readings		$\bar{X}$[1] uptake $\mu\ell\ O_2\ g^{-1}\ hr^{-1}$
3/16	1.84	4		22.8
	1.43	4		28.5
	1.70	4		22.9
3/19	1.44	4		27.0
	2.00	4		36.6
	1.70	4		42.7
	1.92	4		42.2
3/21	1.61	5		9.3
	2.13	5		20.3
4/3	2.60	3		19.8
4/5	2.10	6		9.4
	1.80	6		16.0
	2.55	6		19.0
4/6	2.03	4		32.8
	2.15	4		30.1
	3.04	4		39.7
4/16	1.50	5		23.2
			mean	25.7
			S.D.	10.2

[1]Arithmetic mean.
[2]Standard deviation.

TABLE 7

Oxygen Consumption and Carbon Dioxide Evolution of *Crangon dalli* at 4.5°C

Exp. Date	Pressure (atm)	moles O_2 uptake g^{-1} hr^{-1}	moles CO_2 evolved g^{-1} hr^{-1}
3/16	.970	9.60×10^{-7}	7.75×10^{-7}
		1.21×10^{-6}	9.70×10^{-7}
		9.74×10^{-7}	7.79×10^{-7}
3/19	.977	1.15×10^{-6}	9.25×10^{-7}
		1.56×10^{-6}	1.25×10^{-6}
		1.82×10^{-6}	1.46×10^{-6}
		1.81×10^{-6}	1.45×10^{-6}
3/21	.980	4.00×10^{-7}	3.19×10^{-7}
		8.7×10^{-7}	6.98×10^{-7}
4/3	.975	8.47×10^{-7}	6.77×10^{-7}
4/5	.971	4.00×10^{-7}	3.20×10^{-7}
		6.81×10^{-7}	5.45×10^{-7}
		8.09×10^{-7}	6.47×10^{-7}
4/6	.964	1.39×10^{-6}	1.11×10^{-6}
		1.27×10^{-6}	1.01×10^{-6}
		1.68×10^{-6}	1.34×10^{-6}
4/16	.984	1.00×10^{-6}	$.80 \times 10^{-6}$

Sample calculation: $Pv = nRT$; P = pressure in atmospheres the day of the experiment
R = .0821 liter atm/°K mole
T = 277.5°K
v = volume O_2 uptake
n = number moles O_2

$$\frac{(.974)(2.57 \times 10^{5} \text{ liter})}{(.0821 \text{ liter atm/°K mole})(277.5°K)} = 1.10 \times 10^{-6} \text{ moles}$$

Respiratory Quotient of .8: $(1.10 \times 10^{-6})(.8) = 8.81 \times 10^{-7}$ moles CO_2

$(8.81 \times 10^{-7}$ moles CO_2 g^{-1} hr^{-1} (44 g CO_2 $mole^{-1}$) =
3.88×10^{-5} g CO_2 g^{-1} hr^{-1}
or 1.05×10^{-5} g C g^{-1} hr^{-1}.

DISCUSSION

Virtually all of the prey categories and the gut contents observed have their origin on the bottom. Exceptions such as diatoms, chaetognaths, and fish remains are best explained as dead organic matter (detritus) which has its origin in the water column and has settled to the bottom. In some areas of lower Cook Inlet (Kachemak Bay) up to 12% of primary production in the water column fluxes rapidly to the benthos (Larrance, 1979). Fish remains in crangonid gut contents clearly are a result of dead animals being ingested on the bottom. The large number of food categories (60) is a reflection of the feeding style of these animals. However, it is noteworthy that some of the categories observed in this report may be of limited value as food. A number of polychaete occurrences listed in Table 1 were based on setae identification, not on whole worms. Setae themselves would be of limited food value. Identification of diatom tests in gut contents may bias frequency of occurrence information in that their value as food is questionable. Results of the present study of Cook Inlet crangonids indicate they are clearly generalists employing an opportunistic strategy, that is, eating whatever is available. Similar findings with regard to prey and habits were noted by Wilcox (1974) for an east coast crangonid. From the results of a study of *Crangon septemspinosa*, he concluded they fed on the

bottom and would eat virtually anything. He noted that variation in gut contents may arise from availability of foods, not necessarily preference. That author considers *C. septemspinosa* an omnivore and agrees with an earlier classification (Price, 1962) of the animal as a secondary consumer. It is significant that through direct consumption and as a predator on other detrital feeding organisms (i.e., secondary consumer) Cook Inlet crangonids are dependent on the sediment-detrital food system for their energetic needs.

The observed high frequency of sediment ingestion in the present report is consistent with the study of Wilcox (1974). However, sand was considered to constitute only 4% of the total volume of *Crangon septemspinosa* contents. In Cook Inlet crangonids, inorganic sediment (which includes sand) constituted an overall average of 55.75% (dry weight basis) of the stomach contents after KOH digestion of organic matter. A high percentage contents of sediment in Cook Inlet pandalid shrimps and hermit crabs has been similarly noted (Rice *et al.*, 1980; Feder *et al.*, 1980). Although volumetric estimations were not done in the present study, sediment constitutes a larger fraction of the contents of Cook Inlet crangonids than that found in *C. septemspinosa*. The percent dry weight sediment component was consistently high with two exceptions noted above. These two values may reflect experimental error in that their determination was the first done in the laboratory. It is again noteworthy that the method employed for estimating sediment in this report can be expected to underestimate the actual amount present.

However, this is probably less than volumetric analytical error, considering the rather small size of the animals and their stomachs.

Microbial biomass estimates of Cook Inlet sediments in the present study are in agreement with other recent studies. Atlas (1979) reports 6.4×10^8 cells ml^{-1} from direct counts of sediment samples in inner Kachemak Bay. In Kamishak Bay 3.7×10^8 cells ml^{-1} were observed. Viable counts of these sediments ranged from 4.4×10^6 to 1.8×10^6 CFU g^{-1} units respectively. Viable counts are typically less than direct counts because direct counts include non-viable cells and some bacteria present in the sample may be unable to grow on the media used for the viable count analysis. Griffiths and Morita (1979) report that microbial activities of Cook Inlet sediments were highest in the Kamishak and Kachemak Bay areas. Microbial activity values from 58 ng glutamate g^{-1} hr^{-1} (Kamishak Bay) to 380 ng glutamate g^{-1} hr^{-1} (inner Kachemak Bay) were reported in that study.

Total carbon values of Cook Inlet samples are likely reliable and realistic. However, calculation of organic carbon from total carbon by the method employed increases the potential for error. The organic carbon value for Station 62A was high ($\sim$ 10 mg g^{-1} sediment) while the microbial carbon value was extremely low (< 1 mg g^{-1} sediment). It is noteworthy that large amounts of clam shells ($CaCO_3$) and significant fresh water runoff are found in this area, potentially influencing the inorganic carbon fraction relative to other stations. The presence of such carbon sources on some stations (62A) may in part explain the large difference (relative to other stations such as PMEL 7) between bacterial

carbon values and organic carbon values. Yet, in general, as a means of relative comparison between stations the method is probably acceptable. The contribution of bacterial carbon is potentially significant from a nutritive standpoint. Certain forms of detrital carbon are thought to be of limited value to organisms due to their refractory quality or high C:N ratio.

Respiration rates of *Crangon dalli* in this study were somewhat lower than other crangonids and crustaceans. Hagerman (1970) reported oxygen consumption rate of *Crangon vulgaris* at 6°C to range from 100-200 µl O_2 g^{-1} hr^{-1}, depending on size and salinity. The crab, *Uca* (weight 2 g) at 12°C showed oxygen uptake of 45 µl g^{-1} hr^{-1} (Lockwood, 1967). The average resting rate of *C. dalli* in the present report may be somewhat high due to the influence of the method on the animal's behavior. It is possible that the rate observed here may approach that of active or feeding levels. The carbon flux figure, 1.05×10^{-5} g C g^{-1} hr^{-1} calculated from respiration rates above, can be used to compute carbon demand per day for the average adult shrimp. Thus, a 2 g adult *Crangon dalli* at 4.5°C would need .5 mg carbon in a 24-hour period.

The relationship of the caloric value of ingested sediment with the metabolic needs of the animal is interesting. Additionally, sediment-detrital quality and feeding habits are potentially a function of the oceanographic conditions found at the various stations. Table 8 summarizes the various parameters in this context. For these calculations it was assumed two gut loads were processed daily. Wilcox (1974) reported

TABLE 8

Relationship of Oceanographic Conditions with Sediment Quality and Maximum Sediment Contribution to the Energy Budget of *Crangon* sp.

Station	g m^{-2} *Cangon*	Oceanographic conditions and rate carbon delivered to bottom	Sediment organic C (mg C g^{-1} sediment)	Bacterial carbon (mg C g^{-1} sediment)	$\bar{X}$ amount sediment in gut contents (g)	Daily % Energy[1] Available to *Crangon*[2]	
						Total organic	Bacterial only
PMEL 7	.012	gyre system, productive waters; sediment rich, 60 g C m^{-2} (4-month period)	4.29-10.35	4.8 -5.8	.0025	4.3%-10.4%	4.8%-5.8%
40A	.008		14.53	.001 -1.23[3]	.001	5.8%	<.5%
37	.18		7.77-7.96	5.1 -6.2	na	-	-
PMEL 1	.022	smaller gyre system suggested, sediments fine, some glacially derived; 40 g C m^{-2} (4-month period)	5.94-6.31	2.47 -4.4	.003	7.1%- 7.6%	3.0%-5.3%
62A	.05		10.09-11.66	.18 - .53	.004	16.1%-18.6%	.3%- .8%
PMEL 4	.009	no evidence of gyre, strong currents, course sediments; 17 g C m^{-2} (4-month period)	1.04-1.53	.0003-.3[4]	na	-	-
53	.014		5.24	.5	.007	14.8%	1.4%
Maximum potential - highest values from all categories			14.53	6.2	.007	40.1%	17.4%

[1]Average daily need based on calculated value of .5 mg C/24 hours
[2]Process two average gut loads per day
[3]Based on viable counts (.001), estimate 1.23 if computed from probable number of direct counts
[4]Based on viable counts (.003), estimate 0.3 if computed from probable number of direct counts

a gut transit time of 6-12 hr in *Crangon septemspinosa*. Hence, the average amount of sediment in the gut contents was doubled in the computation. Two general relationships are noted.

(1) There is a relationship of sediment organic and bacterial carbon values with oceanographic conditions and sedimentation rates. The effect of productive waters, gyre systems, and rapid delivery of carbon to the bottom (PMEL 7, 40A, 37) is reflected in higher carbon values in the sediments. Stations PMEL 4 and 53 are in extreme contrast showing impoverished sediments, with Stations PMEL 1 and 62A somewhat in the middle of the two groups of stations.

(2) Lower carbon values appear to result in more sediment consumption by *Crangon*. Stations where sediments were richest (40A, PMEL 7) showed the lowest average amount of sediment in the gut contents. On stations with poorer sediments (e.g., 53) *Crangon* is observed to ingest more sediment. Although these relationships are not totally clear cut, the general trend is evident, and would be clarified with further sampling.

With respect to energy potentials on stations examined, total sediment organic carbon may represent from 4.3% (Station PMEL 7) to 18.6% (Station 62A) of the animal's daily metabolic needs. Further, the bacterial fraction alone could constitute from .5% (40A) to 5.3% (PMEL 1) of the energetic requirements. Bacterial carbon constitutes a small fraction of the sediment organic carbon pool on some stations (40A) and a significant portion of that pool on others (PMEL 1, 7). For example, on Station PMEL 7, bacteria constitute nearly 50% of the organic pool, while nearby on Station 40A bacterial carbon is a negligible fraction

of the organic pool. A hypothetical maximum potential was calculated using the highest values from all categories. In this instance, total sediment organic carbon could represent as much as 40% of the animals daily needs with 17% of that coming from bacterial sources.

Observed percent energy potentials from sediment sources represent a sizeable contribution to the energy budget of *Crangon*. However, it is noteworthy that these contributions in themselves are insufficient for growth and reproduction. Thus, these estimates are consistent with the opportunistic scavenging and predatory behavior observed in Cook Inlet crangonids.

Due to the limited data, no discussion of seasonal effects or nitrogen content of sediments is included. However these two parameters are potentially important. Seasonal production may well affect the quality of food and sediment available to detrital feeding animals in the benthic community. The carbon/nitrogen ratios of potential food sources is an important factor in determining ingestion rates.

Summary and Conclusions

The nature of crangonid feeding habits is interesting and suggests a unique adaptation. Feeding behavior and the amount of sediment ingestion are related to the dynamics of the system. Prey avialability and food resources in the nearshore benthos are quite variable. Shrimp will ingest whatever prey is available in large amounts. Under impoverished conditions accidental or deliberate ingestion of sediment and the apparent ability to utilize affiliated carbon sources enhances their

nutritive intake. A low metabolic rate and sedentary habits serves to reduce caloric needs. Their feeding habits indicate that if hydrocarbons were to become associated with sediments, they would be ingested by *Crangon*. Topics worthy of consideration for future research include examination of sediment organic carbon quality and resuspension of bottom sediments.

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APPENDIX

ASSIMILATION OF BACTERIAL CARBON BY CRANGONID SHRIMP

PREFACE

Attempts to measure assimilation of bacterial carbon by crangonid shrimp are described in the following section. A great deal of interest is currently centered on this topic. Many investigators have attempted to measure assimilation of carbon from bacteria by using radiolabeled bacteria (Adams and Angelovic, 1970; Kofoed, 1975; Rieper, 1978). Although methodological problems exist with this approach, most authors agree that the radiolabeling approach has the necessary sensitivity and is experimentally feasible.

In the experiments described here, the primary goal was to see if crangonid shrimp could digest bacteria and assimilate released carbon by ingesting sediment or detritus enriched with 14-C labeled bacteria. A secondary goal was, if possible, to determine amounts and rates of assimilation. This second interest was important if the significance of bacterial carbon in the diet of these shrimps was to be addressed. However, this second goal complicated the methodology; it was more difficult to determine rates than simply to look for the appearance of label in the body tissue of the shrimps.

Such investigations can prove time consuming, expensive, and experimentally difficult. Constraints on this research existed due to the format under which it was to be conducted. Yet, the question was interesting and was of value both for its intrinsic worth as well as an educational experience for this author.

INTRODUCTION

The use of radioisotopes in studies of detrital food systems has grown both in usage and in sophistication. Detrital utilization studies (Tenore, 1975, 1977), carbon budget studies (Kofoed, 1975), assimilation studies (Adams and Angelovic, 1970; Cummins, 1973), and bacterial assimilation studies (Rieper, 1978) are but a few reports in the literature which reflect the growing use of radioisotopes and their applications in investigations dealing with detrital food systems. Although they are not equally useful in all systems (Conover and Francis, 1973), radioisotopes possess the necessary sensitivity and experimental flexibility to be a powerful research tool. In recent years, advances in areas such as liquid scintillation counting, and increased availability of a wide variety of radioisotopes has made the use of isotopes even more attractive.

The potential of bacterial carbon as energy source for crangonid shrimp in lower Cook Inlet, Alaska has been alluded to in this report. Yet the significance of this carbon source can not be estimated without evidence that these shrimp are in fact able to digest bacteria and assimilate released carbon compounds. Other investigators have shown that related shrimp can survive, and indeed grow on a diet of bacteria (Wilcox, 1974). Certain prawns (*Metapenaeus*) are believed to utilize bacteria as a food source in their natural diet (Moriarty, 1978). However, there were no studies on Alaskan crangonids nor were there any estimates of assimilation efficiency or rates of uptake of carbon

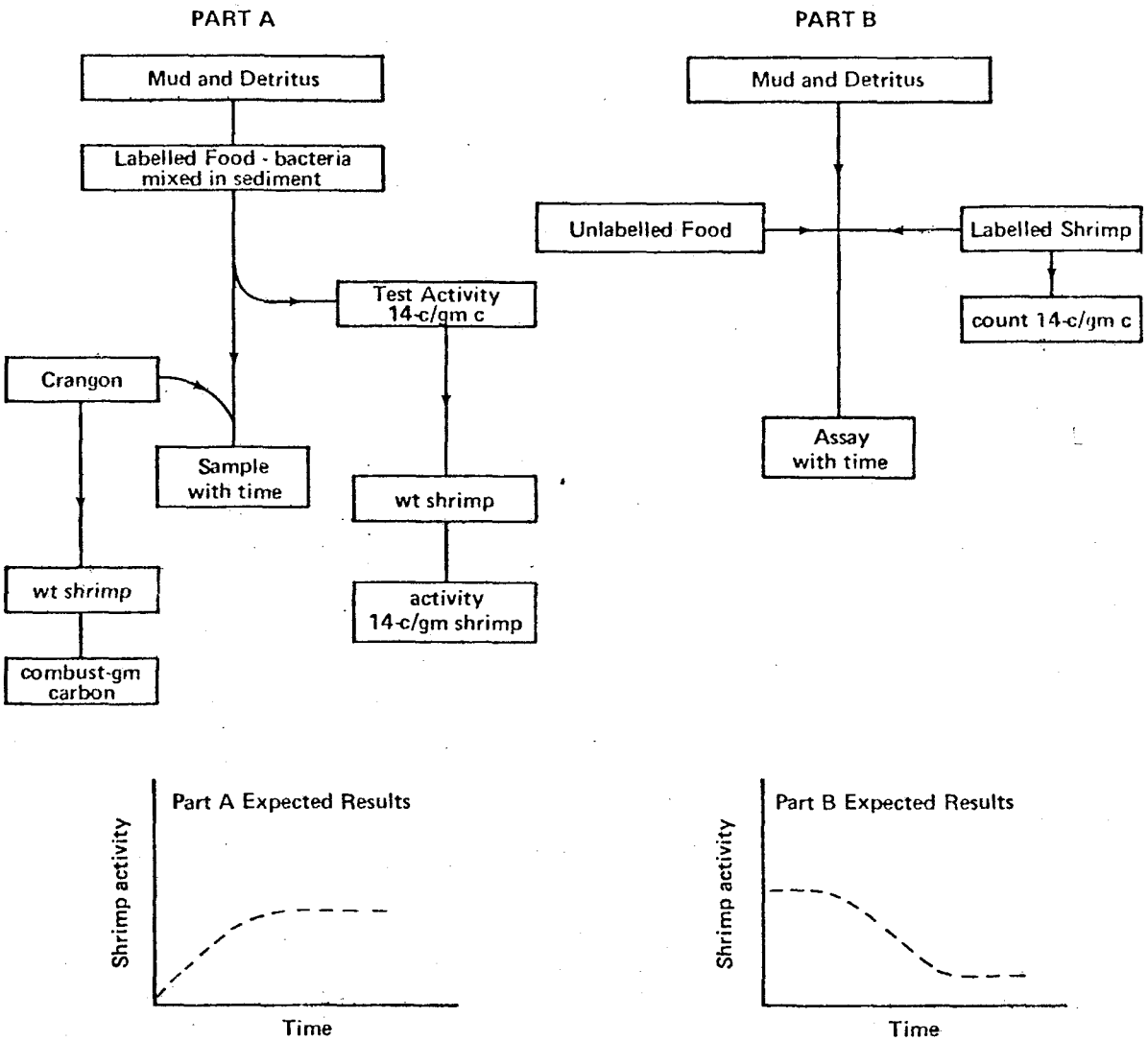
derived from bacteria. Further, there may be a difference between rates of assimilation of bacterial carbon from a "sediment-detrital" slurry source, and from massive amounts of bacteria spun into a pellet and fed directly to shrimp. Hence the goals in the current investigation were on two levels: (1) to answer the very basic question of simply whether or not Alaskan crangonids could assimilate bacterial carbon and (2) providing the answer to 1 was affirmative to determine the rate and efficiency of assimilation.

Appendix Figure 1 depicts the overall experimental approach as originally conceived and presented in my proposal for graduate research outline. The two phases of the work were intended to enable me to calculate carbon uptake rate for the shrimp. Thus, the work as outlined, if successful would answer both of the questions posed above. However, after further consultation with Dr. D. Holleman and Dr. M. J. Klug, modifications to the procedure were incorporated in an attempt to simplify the approach. The opinion was that the experiments as proposed would be much too difficult and complicated and time consuming to be carried out under the existing format.

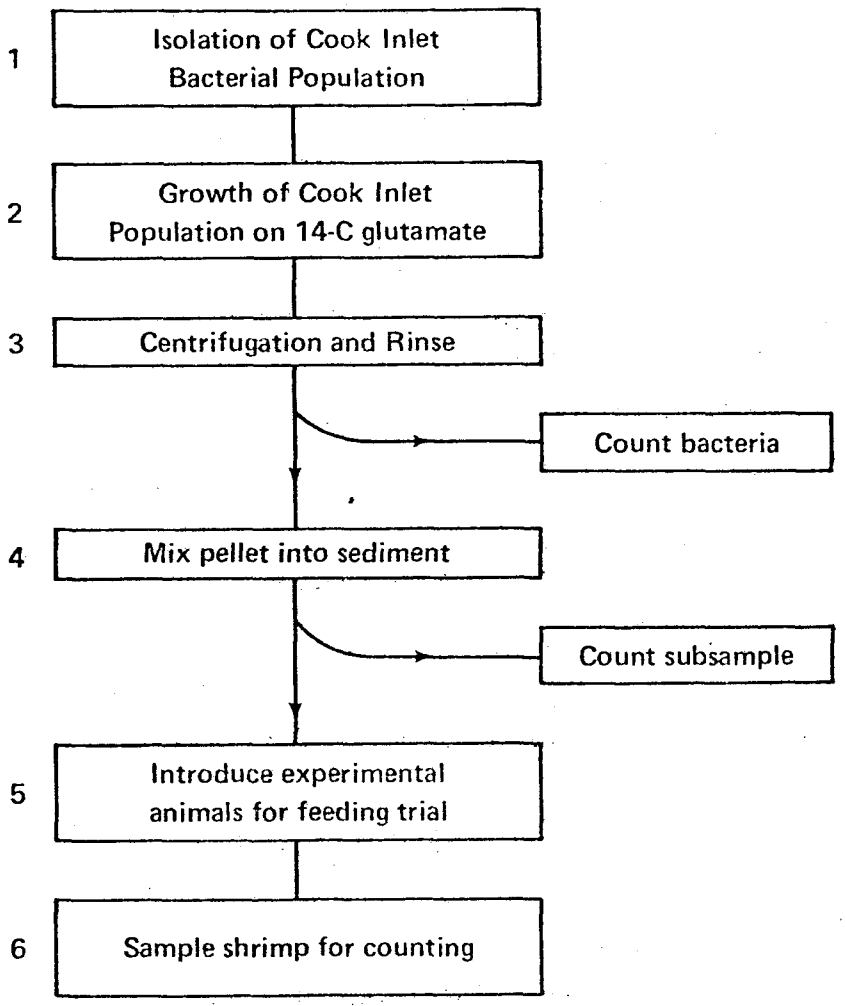
METHODS

Labeled Bacteria Approach

Appendix Figure 2 depicts a flow diagram of the first experimental method employed in this investigation. In the interest of simplicity and in an attempt to obtain an answer to the more basic question of



Appendix Figure 1. Original conception of radioisotope experiments. In part A the animals are on a radioactive food source. In part B radioactive animals are on an unlabeled food source. The graphs under expected results merely indicate the general trend expected. The purpose of the loading and unloading experiments was to be able to calculate carbon uptake rate by difference.

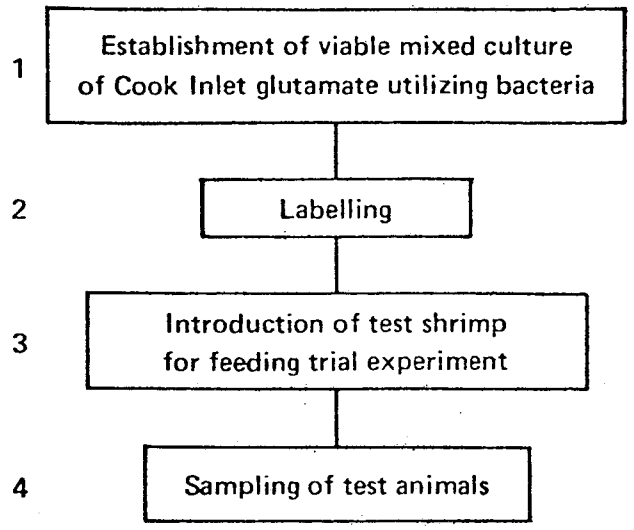


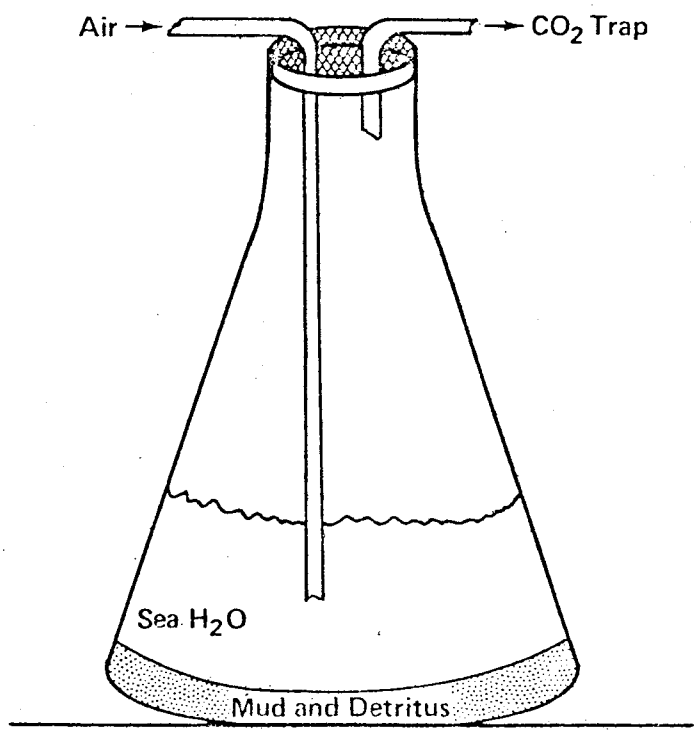
Appendix Figure 2. Previously cultured labeled bacteria approach.

bacterial carbon assimilation, the loss of label portion of Appendix Figure 1 was eliminated and a simple "loading" experiment was performed. This method consisted of 6 steps. In Step 1, a mixed culture of Cook Inlet bacteria was established by enrichment on a glutamate containing media. In this way, uptake of radiolabeled glutamate would be facilitated (Step 2). After centrifugation and rinsing (Step 3) bacteria was counted, and the pellet was mixed into a sediment detrital slurry to uniformity (Step 4). A subsample of this final mixture was also counted for 14-C activity. Step 5, then, was the introduction of the experimental animals, usually shrimp, although the technique was applied in the first run to *Macoma* clams. Step 6 was the sampling and counting of the experimental animals.

Closed Mini-Ecosystem Approach

Appendix Figure 3 depicts the methodology used in a second series of experiments. In this system a more dynamic approach is used, where a mini-ecosystem is set up and growth of bacteria and feeding trials of the shrimp are both carried out in a closed system. At the outset, it was felt that if this system worked properly, it would be easily expanded so that label unloading of tissues could be observed, hence, computation of rate of assimilation and turnover would be facilitated. Further, this system was believed to better approximate the natural environment where the shrimp are found. Bacteria should be attached and viable if this method is correct, and shrimp would then be required to digest them off the sediment and then void the sediment.





Appendix Figure 3. Experimental set up for closed mini-ecosystem approach. The test chamber is a specially modified 6 L Ehrlenmeyer flask.

Four principal steps were involved in this method. First, a culture of Cook Inlet glutamate utilizing bacteria was established in a large airtight flask (Step 1). Sediment was enriched with amino acids to prompt growth of potential glutamate users. Then, in Step 2, 14-C labeled glutamate was introduced. At this time the system was sealed and evolved gases from the chamber were passed through a CO_2 trap. The solution in the trap was monitored and counted so that uptake and respiration of the label by the bacteria could be detected (see sample preparation, below). After it was determined that the bacteria population was actively growing, incorporating and respiring label, the test animals were introduced for the feeding trial (Step 3). A sufficient number of shrimp were used to allow for non-feeding individuals and individual variation. The final step then was the sampling and counting of the animals with time (Step 4) (see sample preparation).

Preparation of Standards, Samples, and Scintillation Information

Appendix Table 1 summarizes information concerning the scintillation cocktails and methods used for the various types of samples. A series of quenched standards for each type of sample were prepared and counted. It was found that quenching of the various samples differed significantly (i.e., slopes of counting efficiency versus external standards ratio differed), thus making it necessary to prepare such a series for each type of sample. These standards could then be used in the analysis of samples from the experiments.

APPENDIX TABLE 1

Sample Preparation and Liquid Scintillation Information

Type of Sample	Amount Sampled	Ingredients of Cocktail and Treatment
Tissue homogenates	1/4 homog-.8 ml	2.0 ml protosol; digest at 50°C overnight; .1 ml H_2O_2, 15 ml LSC[1]; Efficiency range 50-80%; Slope of quench curve 10.6.
CO_2	-	2.0 ml protosol; 8 ml LSC, 8 ml methanol; Efficiency range 40-70%; Slope of quench curve 11.8.
Seawater	1 ml	.15 ml Aquasol; Efficiency range 80-90%; Slope of quench curve 2.96.
Sediment	1. - .5 ml	Same as tissue homogenates; Efficiency range 50-80%; Slope of quench curve 10.2.

LSC information: Bechman LS 100C counter with external standards ratio capability - count time, 10 min. each.

[1]LSC = Omnifluor/toluene; 4 g/liter
Quench curve = counting efficiency (cpm/dpm) versus external standards ratio (ESR)
Omnifluor, Protosol available from New England Nuclear,
label was L-Glutanic Acid [^{14}C(U)] - New England Nuclear. Lot 1152-038
Specific activity 296 mCi/m mole.

Samples for counting were collected in the following manner. Shrimp were killed, rinsed, and the tail section removed. The shell was removed and the tissue again rinsed. The intestine, lying along the ventral surface of the tail section was carefully dissected out and the tail tissue again rinsed. The tissue was then weighed and a 1:4 homogenate was prepared in a Waring blender fitted with a micro-cup. A .8 ml subsample was then removed for the protosol treatment and counting. In some cases, the intestines and gills of the animal were also counted. Clams were killed, rinsed, and the gut, intestine, and gills removed. The remaining tissue was again rinsed, removed from the shell, weighed and used to prepare a 1:4 homogenate as above. A control animal was also in the test chamber in a cloth-mesh enclosed vial so that it would not be able to feed.

Sediment samples were siphoned off the surface of the substrate with a pipette and placed directly in a scintillation vial and treated with Protosol, as were the tissue homogenates. Incubation of sediment and tissue samples at 50°C with Protosol greatly facilitates solubilization. After the samples are digested, bleaching with peroxide helps in the reduction of color quenching.

Seawater samples of 1 ml were taken from the experimental chambers using a pipette and counted in Aquasol. One such sample was taken, counted, then acidified (.1 N HCL) and recounted. It appeared that most of the activity ($\sim$ 90%) in the water was in the form of bicarbonate.

CO_2 samples were taken from a manifold of scintillation vials which contained the cocktail described in Appendix Table 1. Specially prepared

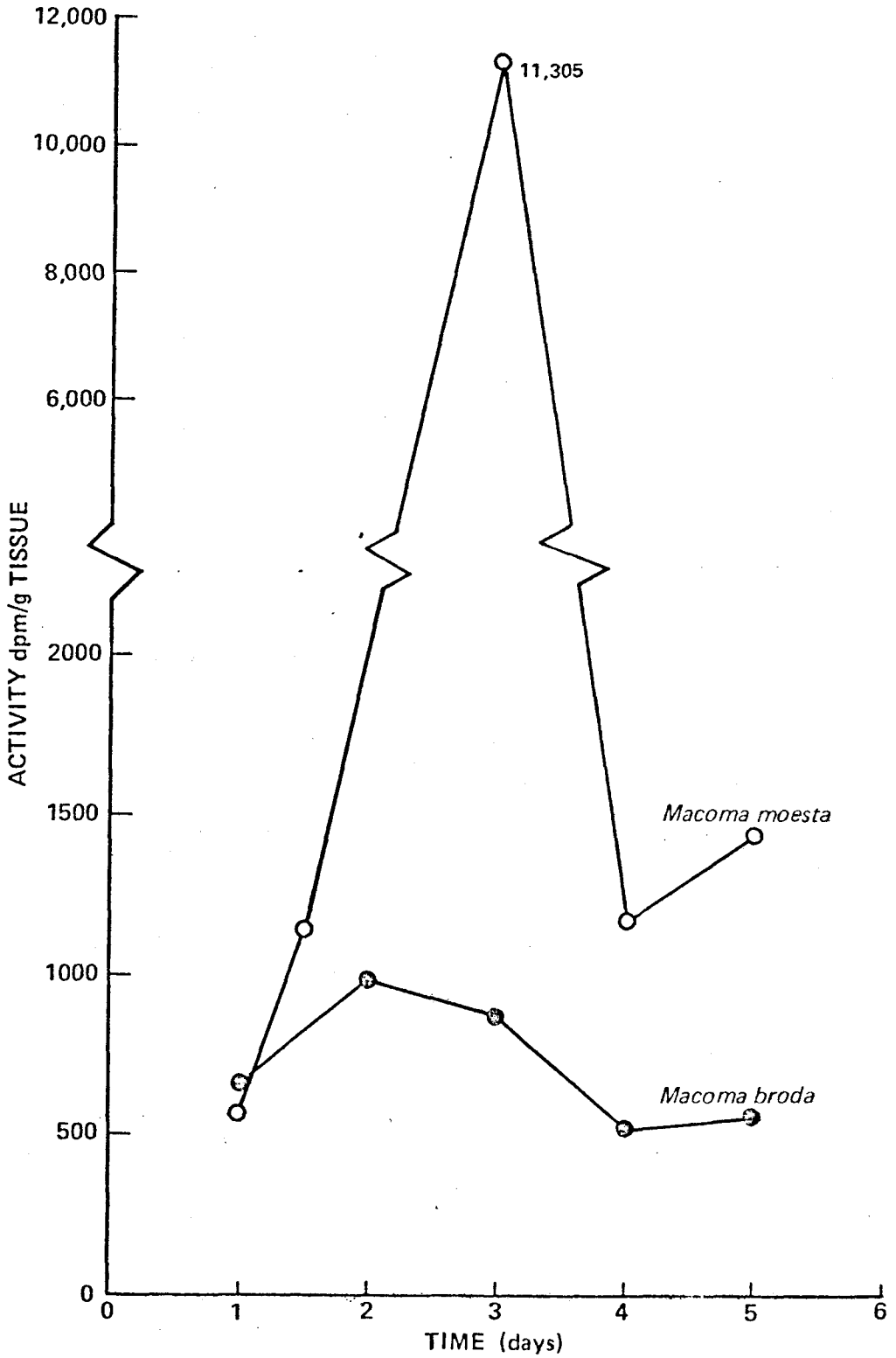
caps for the vials were drilled, fitted with glass tubing, and then sealed with silicone so that air and gases from the experimental chamber (air-tight) would pass through the manifold and CO_2 trapping cocktails. The vials were connected in series so that the evolved gases would pass through a total of six vials before going through a final trap. It was determined that more than 90% of the counts evolved were trapped in the first vial, and that the last or sixth vial in the manifold trap set-up showed only background levels of activity.

RESULTS

Previously Cultured Labeled Bacteria Experiments

Appendix Figure 4 displays the results of the first experiments in which clams of the genus *Macoma* were allowed to feed on a sediment-detrital mixture containing bacteria which had been previously grown on a medium containing 14-C glutamate. As evidenced by the graph, clam tissue showed a maximum of activity 2-3 days after the experiment began. The general shape of the curves for the two species of clams was similar, although the actual amounts of activity differed somewhat. The activity observed in the clam tissue homogenates was low relative to the bacterial culture broth, which when counted showed 2×10^6 dpm ml^{-1}. However, the final sediment-detrital mixture showed 7×10^3 dpm ml^{-1} when the test animals were introduced.

The experiment using culture grown labeled bacteria as food for crangonid and pandalid shrimp proved to be unsuccessful. Although



Appendix Figure 4. Activity of clam tissue homogenates.

labeled bacteria were successfully grown, the test animals did not feed, and some died. The experiment was carried to completion anyway. It is of interest that non-feeding animals typically showed low activities throughout the experiment, with tissue activities as well as activity of the water staying near background levels. The sediment-detrital mixture was found to have an activity of 5.8×10^4 dpm ml^{-1}.

Closed Mini-Ecosystem Experiments

Results of the first attempts at the closed system assimilation experiment are summarized in Appendix Tables 2 through 4. It is evident from Appendix Table 2, $^{14}CO_2$ evolution, that the bacteria readily incorporated the 14-C glutamate label. Further, after mixing the bacterial growth mixture into the sediment-detrital mixture, the final substrate upon which shrimp were placed for the feeding trial, showed high levels of activity (3.5×10^4 dpm ml^{-1}). The sediment activity varied with time as indicated in Appendix Table 3.

Activity data for individual shrimp homogenates are graphed in Appendix Figure 5. Activity appeared rapidly in shrimp tail tissue and then tapered off to lower levels by the end of the experimental period. Gills of these same shrimp showed very high activity levels (to 42000 dpm). These animals were observed to feed during the experiment. Appendix Table 4 displays an account of the label for the experiment.

A second experiment, utilizing the same approach as the one above, but with more animals was attempted. Again bacteria incorporated label (7×10^5 dpm in 24 hr) and the substrate for the feeding trial showed

APPENDIX TABLE 2

$^{14}CO_2$ Evolution from Bacterial Growth

Experimental Time (hrs)	DPM/6 vials (CO_2 trap)
6.75	6.43×10^5
15	2.43×10^6
18.5	3.55×10^6
26.5	4.32×10^6
Bacterial growth-total $^{14}CO_2$ evolved	
$^{14}CO_2$ evolved during feeding trial	1.43×10^6
Total CO_2	

APPENDIX TABLE 3

Activity of Sediment Samples During Feeding Trial

Experimental Time (hr)	Activity (dpm ml^{-1})
T = 0	3.50×10^4
4.25	9.88×10^4
12.50	1.34×10^5
15.75	4.98×10^4
20.00	7.08×10^4
25.00	1.75×10^5
29.00	1.13×10^5
38.00	2.96×10^4

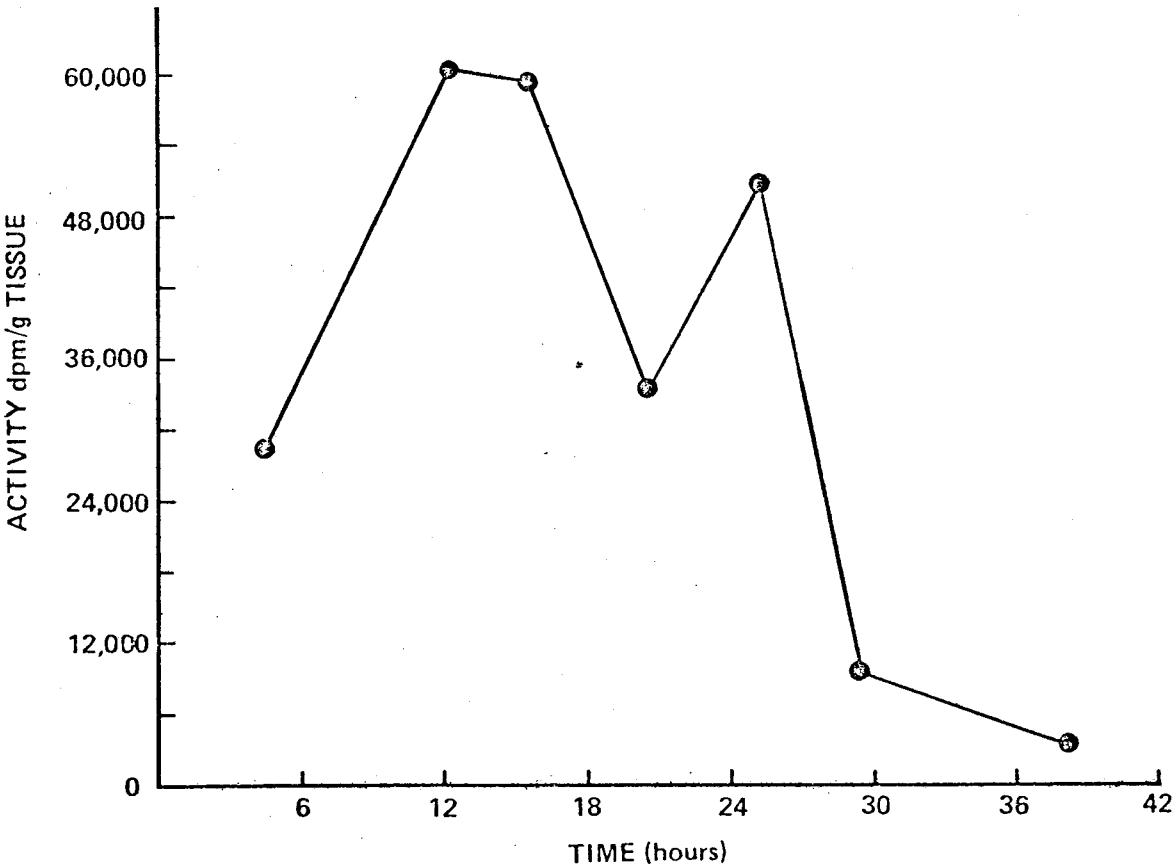
APPENDIX TABLE 4

14-C Label Account for Mini-Ecosystem Bacterial Growth and Shrimp Feeding Trial

Introduce 20×10^7 dpm

CO_2 evolved - bacterial growth	1.09×10^7 dpm
CO_2 evolved - feeding trial	1.43×10^6 dpm
Total label in all shrimp tissues (includes gills, etc. for all shrimp)	8.10×10^5
Total	1.31×10^7 dpm

Remainder (6.86×10^6 dpm) still in sediment substrate at completion of experiment.

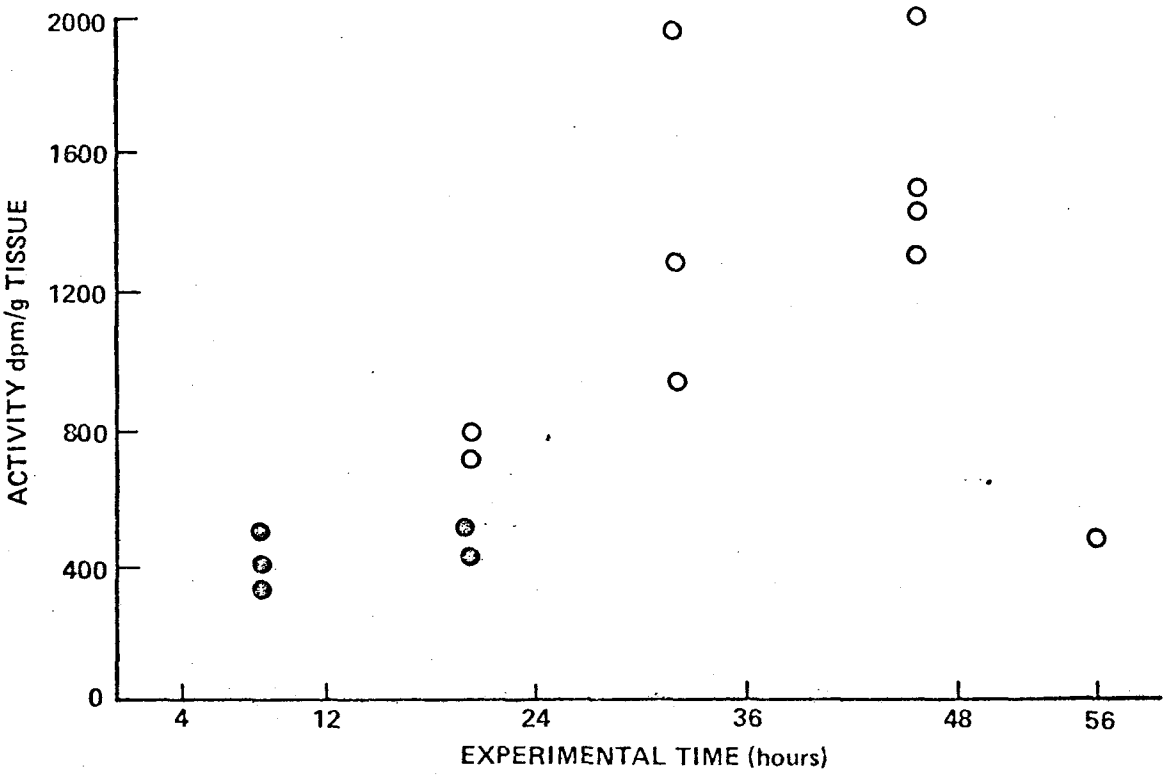


Appendix Figure 5. Activity of individual *Crangon dalli* tissue homogenates.

good levels of activity (4×10^4 dpm ml^{-1}). However, shrimp death and problems with the system resulted in failure of the experiment. Several of the experimental animals died and it was necessary to restart the experiment with new ones on the same substrate. These animals in most cases did not feed. Appendix Figure 6 shows the activities of shrimp tissue homogenates for this experiment. It is noteworthy that up to 2000 dpm g^{-1} tissue were recorded for animals that did not feed and had empty guts at the time of collection. Problems with the aeration system resulted in resuspension of the substrate.

DISCUSSION

Results have been presented which suggest that *Macoma* spp. clams assimilated bacteria which had been previously grown on a radioactive label. Although activity levels of the clams were low, the general shape of the curves suggests that label was assimilated, then metabolized, resulting in a loss of activity in the tissues as suggested in the outline (Appendix Figure 1). The use of the labeled bacteria in this experiment appeared to be a satisfactory method and to be relatively free of complications. Although the shrimp experiment using the previously cultured labeled bacteria approach did not work the methods seem satisfactory. More specifically non-feeding animals did not pick up activity, nor did the water or other pools in the experimental set up. Non-feeding animals and death of experimental animals are hazards of experimental biological systems. Hence, this approach appears to be a workable one provided



Appendix Figure 6. Activity of *Crangon dalli* tissue samples. Darkened points are the values of shrimp tissue homogenates which were taken from animals which had observable gut contents. All other points are from shrimp which did not feed.

that activity levels can be adjusted by modifying the amount of label introduction, volume of sediment-detrital mixture and numbers of experimental animals to be dealt with. In the clam experiment, too much dilution of the pregrown bacterial broth by the sediment-detrital mixture took place because the volume of the sediment and the experimental set up was too large.

Results of the mini-ecosystem approach were ambiguous. The first experiment suggested that crangonid shrimp did indeed assimilate the bacteria which had been labeled. High levels of activity were observed after 18-24 hours of exposure to the food source. Again the level of activity began to drop with time as above. This drop in activity was similarly noted for the sediment-detrital food source.

The second experiment using this approach left some doubt as to what was actually being assimilated by the shrimp. In this case animals which had not fed showed activity. This suggests that they were absorbing label from some other source. Further, the problems encountered with this approach were much greater than with the use of the pregrown labeled bacteria. Glassware, tubing, aeration, etc. contributed to the overall complexity of the set up and made for problems during the actual running of the experiment. Resuspension of labeled substrate was a big problem which led to the demise of the control. Labeled substrate found its way into the control chamber thus making activity available to the control animal. However, even if the shrimp showed such levels of activity as these (2000 dpm) it is still suggested from the first experiment that they were absorbing additional label, presumably from bacteria.

The failure of the second experiment poses an interesting question: of what significance is the assimilation of carbon from dissolved sources? This question has frequently been raised for certain types of detrital feeding animals. Recent work has demonstrated that detrital feeding fishes are capable of assimilating detrital non-protein amino acids (Bowen, 1980). Crangonids, residing in a rich organic slurry of sediment and detritus as they do, might be able to take advantage of dissolved organics such as amino acids.

In summary, the previously cultured labeled bacteria approach may be of more value in addressing the simple question of whether or not an animal can assimilate bacterial carbon. The closed system approach as employed here led to problems in interpretation of the results as noted by Conover and Francis (1973). It appears these animals are able to assimilate bacterial carbon, and that additionally they may be able to assimilate dissolved sources of carbon and nutrients. However, from these experiments it is not certain how much occurs from either source.

If further efforts to examine bacterial and dissolved carbon assimilation in these animals were to be initiated, several points are noteworthy in terms of methods. An open flowing seawater system with previously cultured labeled bacteria should be employed. Scintillation cocktails using Biofluor (NEN) should be used for most types of samples with the exception of CO_2. CO_2 can be collected on filter paper soaked in Protosol and counted in the Omnifluor cocktail.

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